

AD-A103 649

STRUCTURE AND DESIGN OF MULTIPOTENT PEPTIDE  
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MEDICINE M E SELSTED 01 AUG 87 N00014-86-K-0525

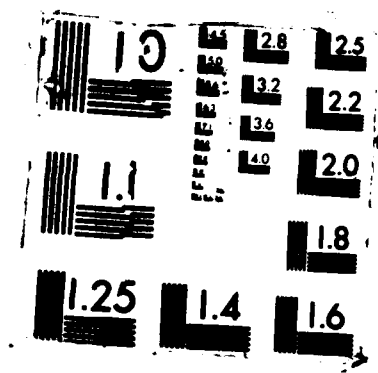
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REPORT DOCUMENTATION PAGE **DTIC FILE COPY**

**AD-A183 649**

2b. DECLASSIFICATION/DOWNGRADING SCHEDULE NA		1b. RESTRICTIVE MARKINGS NA		<b>DTIC ELECTE</b> <b>Aug 24 1987</b> <b>S D</b>	
4. PERFORMING ORGANIZATION REPORT NUMBER(S) NA		3. DISTRIBUTION/AVAILABILITY OF REPORT Unlimited			
6a. NAME OF PERFORMING ORGANIZATION University of California Los Angeles		6b. OFFICE SYMBOL (if applicable) NA		7a. NAME OF MONITORING ORGANIZATION Office of Naval Research	
6c. ADDRESS (City, State, and ZIP Code) Dept. of Medicine, 37-055 CHS UCLA School of Medicine Los Angeles, CA 90024		7b. ADDRESS (City, State, and ZIP Code) 800 N. Quincy St. Arlington, VA 22217-5000			
8a. NAME OF FUNDING/SPONSORING ORGANIZATION Office of Naval Research		8b. OFFICE SYMBOL (if applicable) ONR		9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER N00014-86-K-0525	
8c. ADDRESS (City, State, and ZIP Code) 800 N. Quincy St. Arlington, VA 22217-5000		10. SOURCE OF FUNDING NUMBERS			
		PROGRAM ELEMENT NO. 61153N		PROJECT NO. RR04106	WORK UNIT ACCESSION NO.
11. TITLE (Include Security Classification) (U) STRUCTURE AND DESIGN OF MULTIPOTENT PEPTIDE MICROBICIDES					
12. PERSONAL AUTHOR(S) Michael E. Selsted, Ph.D., M.D.					
13a. TYPE OF REPORT annual		13b. TIME COVERED FROM 8-1-86 TO 8-1-87		14. DATE OF REPORT (Year, Month, Day) 8-1-87	
15. PAGE COUNT					
16. SUPPLEMENTARY NOTATION					
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP	Peptides, microbicides, structure-function, synthetic peptides		
19. ABSTRACT (Continue on reverse if necessary and identify by block number)					
<p>→ A family of antimicrobial leukocyte peptides has been isolated from the neutrophils of several species. I am using the consensus structure of the peptides (known as defensins) as a molecular foundation for generating new antimicrobial peptides by synthetic methods. The synthetic approach is directed by correlating the solution structures of various defensins with their distinctive biological activities. Report consists of a brief description of recent accomplishments.</p>					
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20. DISTRIBUTION/AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION (U)		
22a. NAME OF RESPONSIBLE INDIVIDUAL Dr. Eli D. Schmeil			22b. TELEPHONE (Include Area Code) (202) 696-4760		22c. OFFICE SYMBOL ONR

# STRUCTURE AND DESIGN OF MULTIPOTENT PEPTIDE MICROBICIDES

ONR-N00014-86-K-0525

Michael E. Selsted, M.D., Ph.D.

UCLA Department of Medicine

Division of Hematology and Oncology

Center for the Health Sciences

Los Angeles, CA 90024

## ANNUAL REPORT

August 1, 1987

**Project goal.** The goal of this project is to design novel peptide antibiotics using a naturally occurring family of peptides, now known as defensins, as models. Defensins are 29-34 amino acid peptides which have homologous structures defined by 8 conserved residues. Although the defensin structures are ca. 25% conserved, the variation which occurs among non-conserved amino acids is responsible for their diverse and selective spectra of antimicrobial activities. By correlating specific biological activities of the various defensins, we seek to design custom peptide antimicrobials which will possess activities determined by novel sequences, thereby generating unique and predictable structures. The approach which we have taken has been to compare the primary, secondary and tertiary structure of members of the defensin peptide family. Because of their size, the fact that they are well characterized, and because they are amenable to study by crystallographic and nuclear magnetic resonance methodologies, we have a unique opportunity to establish relationships between polypeptide structure and function using this unique family of biologically active molecules.

### **Recent accomplishments.**

1. Determination of defensin disulfide structure. Each of the defensin peptides contains three intramolecular disulfide bonds. In order to establish the constraints conferred by

the cystines, we sought to determine the disulfide array within a representative member of the peptide family. This was recently completed, and a manuscript describing the findings is in preparation. Briefly, we have found that the disulfide array within one of the human defensins, HNP-2, is formed by a pairing the first and sixth, the second and fourth, and the third and fifth cysteines within the polypeptide chain. We used a novel approach in establishing the disulfide pairs within this defensin, utilizing amino and carboxyl specific tags to determine the pairing of specific cysteine residues. Interestingly, the disulfide array present within the defensins is completely unique, and is distinct from any other known cysteine pairing, e.g., such as those found in the neurotoxins, growth factors, and complement component C9. The establishment of this unique disulfide motif has allowed us to limit the number of potential defensin structures to a relative few.

2. Two-dimensional nuclear magnetic resonance spectroscopy of human and rabbit defensins. 2D-NMR has been used to analyze the solution structures of one rabbit defensin, NP-5, and one human defensin, HNP-1. By methods which are described in detail in an accompanying manuscript proof, the general fold of each of these peptides mentioned above was determined. The structures obtained were derived from data which did not include the disulfide structure which is now known. Interestingly, the location of the disulfide pairs within this defensin are completely consistent with the data obtained by the NMR spectroscopic methods. The NMR approach has generated a family of highly related structures for HNP-1 and NP-5. An example of the structures of NP-5 is shown in Figure 1 below.



Fig. 1. Stereoview of four different NP-5 structures based on distance geometry conversion of 2-D NMR spectroscopic data.

3. Crystallographic analysis of defensins. We have grown diffraction quality crystals of five of the defensin peptides and are currently analyzing datasets obtained by x-ray diffraction analysis of the human defensin HNP-1. One goal is to correlate the structures obtained by x-ray crystallography with those of the NMR analysis.

4. Synthetic defensins. We have employed automated solid phase peptide synthesis to construct a full length synthetic version of the rabbit peptide NP-2. Using a commercially available solid phase synthesizer, we have synthesized approximately 10 mg of this peptide, and have generated native, correctly refolded NP-2 which has been purified to homogeneity by reversed phase high performance liquid chromatography. Physical and chemical characterization of this material suggests that it is indistinguishable from native NP-2, and we are currently assessing its biological activities. The yield NP-2 was approximately 10% of the synthetic product. We are presently determining methods to increase the yield by solid phase synthetic methodology..



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